

Sensitivity pattern of *Neisseria gonorrhoeae* to penicillin and screening for β -lactamase production in Ibadan, Nigeria

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SUMMARY The few reports from Africa on sensitivity determinations of *Neisseria gonorrhoeae* suggest that there is an increasing resistance in the strains, as has been found in other parts of the world. In the current study, the penicillin sensitivities and β -lactamase production of 80 consecutive strains of *N. gonorrhoeae* isolated from cases of acute urethritis in African men in Ibadan were studied. Of these strains, 17.5% had a penicillin minimum inhibitory concentration (MIC) of 0.038 μ g/ml or less and were considered as being 'fully sensitive'. However, 82.5% had an MIC of 0.075 μ g penicillin/ml or more, and were considered as having 'diminished sensitivity' to penicillin. It was thought that this high incidence of relatively insensitive strains was owing to the sequential selection of these strains because of the easy availability and abuse of antimicrobial agents by the general population. Furthermore, 13 strains (16.25%) demonstrated high level resistance with MIC values of over 0.6 μ g penicillin per ml and it is probable that many of the strains will demonstrate multiresistance to the commonly misused antimicrobial agents. Consequently, treatment of patients harbouring these strains may present problems because of financial constraints of the health services in purchasing the appropriate antibiotics. Despite the high level resistance of the strains in an environment of intensive penicillin and ampicillin use, none of the strains studied showed any evidence of β -lactamase production. Nevertheless, continuous surveillance of β -lactamase production by the gonococcus is recommended in the larger medical centres in developing countries.

Introduction

The ever increasing prevalence of antibiotic resistant strains of *Neisseria gonorrhoeae* has been reported from all over the world (Willcox, 1972). Although penicillin still remains the drug of choice in the treatment of acute uncomplicated gonococcal infection, therapeutic failure with this drug is becoming more common. The recent reports of penicillinase-producing strains of *N. gonorrhoeae* questions the use of penicillin in the treatment of gonorrhoea (Ashford *et al.*, 1976; Phillips, 1976).

Despite the lack of information, it is thought that Africa exemplifies the growing trend in penicillin

insensitivity of the gonococcus. This has been confirmed by treatment failures and devastating chronic lesions of the gonococcus in Africa (Osoba and Alausa, 1976). Reports from Africa on the sensitivity pattern of the gonococcus to penicillin have been recorded from Uganda (Arya *et al.*, 1973), Ethiopia (Plorde *et al.*, 1973), Kenya (Verhagen *et al.*, 1971), and South Africa (Finlayson *et al.*, 1974). These reports showed a decreased sensitivity to penicillin ranging from 20 to 80% of the various strains tested.

More recently, one of the earliest strains of β -lactamase-producing gonococci was thought to have originated from West Africa (Phillips, 1976; Arya, personal communication). We therefore thought it worthwhile to study the penicillin sensitivity and β -lactamase production of strains of *N. gonorrhoeae* isolated at the University College Hospital, Ibadan, Nigeria.

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Materials and methods

Strains of *N. gonorrhoeae* were obtained from 80 consecutive cases of acute purulent gonococcal urethritis in African men presenting at the Special Treatment Clinic, University College Hospital, Ibadan, Nigeria. The specimens were obtained by passing sterile cotton-wool swabs into the anterior urethra and plating directly on to Thayer-Martin medium (Oxoid) and chocolate agar. The plates were then incubated in candle extinction jars at 37°C for between 24 and 48 hours. Typical colonies were picked and Gram stained. All Gram-negative cocci were then further identified after subcultures on brain heart infusion agar (BHIA) (Oxoid) by the oxidase reaction and fermentation reaction in serum-free agar sugar medium (Flynn and Waitkins, 1972). Strains were identified as being *N. gonorrhoeae* only if they were oxidase-positive and fermented glucose but not sucrose or maltose.

MINIMUM INHIBITORY CONCENTRATION (MIC) DETERMINATION

Serial twofold dilutions of penicillin G (Crystapen) were added to BHIA cooled to 50°C to give final concentrations of 1.2, 0.6, 0.3, 0.15, 0.075, 0.038, 0.019, 0.01, and 0.005 µg penicillin/ml. The agar was allowed to set and the plates were then dried in an incubator at 40°C for 30 minutes and used on the day of preparation.

Several colonies of the strain to be tested were picked from a 48-hour culture on BHIA and emulsified in saline. The concentration was adjusted visually to a No.05 McFarland standard. This suspension contained between 10^7 and 10^8 organisms/ml.

An even suspension of the organisms was obtained by agitation on a vortex mixer for about 10 seconds. Using a standard platinum loop, 0.001 ml of the suspension was inoculated on to the antibiotic-containing plates. These were then incubated in an extinction jar at 37°C for 18 hours and the tests read as MICs. The lowest concentration of penicillin which completely inhibited the growth of the test organism or caused a decrease of more than 95% in the growth of the organisms was regarded as the MIC for that strain.

A control plate without an antibiotic and a reference strain of *Staphylococcus aureus* with an MIC of 0.036 µg penicillin/ml were included in each test. Reference strains of the World Health Organisation of *N. gonorrhoea* III, V, and VII were obtained from Dr Alice Reyn at the Statens Serum Institut, Copenhagen. Using the above method the MIC from the reference strains did not differ significantly from the figures obtained at the WHO reference laboratory.

CHROMOGENIC CEPHALOSPORIN TEST

5 mg of chromogenic cephalosporin compound 87/312 (Glaxo) were dissolved in 0.5 ml of dimethyl sulphoxide and the solution was made up to 10 ml with 0.1 mol/l phosphate buffer pH 7.0. The solution was used either freshly prepared or after storage at +4°C for not longer than one week. To test for β-lactamase production, the solution was dropped into confluent growth of the *N. gonorrhoeae* strain under test and observed for the development of a red colour both immediately and after a further 30 minutes' incubation at 36°C. A control culture of penicillinase-producing *S. aureus* was used to ensure that the reagent was working satisfactorily each time it was used.

Results

The sensitivities to penicillin of the 80 strains of *N. gonorrhoeae* are illustrated in the Figure. Fourteen strains (17.5%) had MIC values to penicillin of 0.038 µg/ml or less. These were considered as being 'fully sensitive'. Fifty-three strains (66.25%) had MIC values of 0.075 to 0.3 µg/ml penicillin. These we considered to have a 'reduced sensitivity' to penicillin. Thirteen strains (16.25%) had MIC values of over 0.6 µg penicillin/ml. These were considered as being 'highly resistant'.

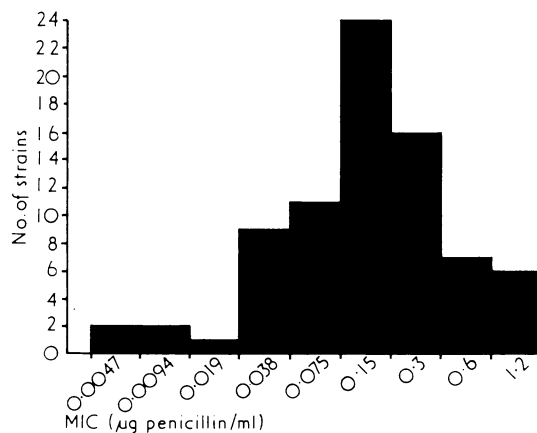


Figure The sensitivities to penicillin of the 80 strains of *N. gonorrhoeae*.

All the highly resistant strains were tested and gave a negative result on the chromogenic cephalosporin test indicating that none of them produced penicillinase.

Discussion

Reports about antimicrobial drug resistance by strains of *N. gonorrhoeae* started to appear in 1944 (Reyn *et al.*, 1958). Since then the impression that there was a progressive increase of gonococcal resistance to antibiotics, especially to penicillin, has been confirmed (Amies, 1969; Willcox, 1970, 1972).

Although the situation in the African region is not as well defined as in other countries, the little information available indicates that the world-wide pattern also applies to this continent. In West Africa, although 99% of 74 gonococcal strains were found to be 'fully sensitive' in 1961 (World Health Organisation, 1963), Clarke (1964) working in Lagos, encountered treatment failure in 21.4% of patients who received procaine penicillin 2.4 megaunits daily for three consecutive days although no sensitivity tests were performed. In Yaunde, Cameroons, Millan and Huet (1970), using a disc diffusion method, reported that 10 of their 18 gonococcal strains were 'sensitive' in penicillin. Osoba (1972), using the disc diffusion technique, found 19 of 185 strains of gonococci isolated in Ibadan were resistant to 1.5 units of penicillin. Three of these strains came from prostitutes taking prophylactic antibiotics.

In Ethiopia, Plorde *et al.* (1973) found 234 (50%) strains from Addis Ababa had an MIC value to penicillin of 0.036 µg/ml or less. Increasing resistance of the gonococcus to penicillin has been demonstrated in East Africa by Phillips *et al.* (1969) who found that 193 (70%) strains were resistant to 0.06 µg/ml penicillin or less, and Arya and Phillips (1970) who reported that 173 (80%) strains showed diminished sensitivity to penicillin.

We found 82.5% of our strains showed diminished sensitivity to penicillin. This is comparable with the East African figures and with 85% reported in the Far East (Keys *et al.*, 1969), but is higher than the British figures of 17.6% (Shannon *et al.*, 1975) and 30% (Shahidulla and Greaves, 1975). It is also higher than the figures reported from Bombay (Moses *et al.*, 1971) and the 70% reported from the USA (Jaffe *et al.*, 1976). The level of gonococcal penicillin resistance in our series therefore ranks as one of the highest in the world. When the figures obtained in the current study are compared with those reported in 1961 (World Health Organisation, 1963) it appears that penicillin-insensitive strains are being rapidly and sequentially selected, particularly during the last 15 years.

Several reasons can be found for this high incidence. The main one is the easy availability and abuse of antimicrobial agents by the general population. It has been shown that by improving the results of

treatment of gonorrhoea by increasing the dosage of penicillin lowers the prevalence of partially resistant strains (Morton 1963; Olsen and Lomholt, 1969). However, because of the inadequate medical facilities and a general misconception of antimicrobial agents by patients, there is reckless self-medication. Not only are antibiotics taken prophylactically after sexual intercourse with casual consorts, but even if symptoms of acute urethritis develop, subcurative and ineffective doses of these agents are taken haphazardly for varying periods before a proper medical opinion is sought.

With a gonorrhoea infection rate of 15.8% among prostitutes and 17% among female hospital patients in Ibadan (Osoba, 1972), and widespread promiscuity, this high rate of penicillin resistance in strains of the gonococcus poses a great economic problem as more expensive antimicrobial agents will have to be used to treat these patients. More alarming still is the evidence obtained in Kenya by Verhagen *et al.* (1971) that the reservoir of relatively resistant strains in a tropical country is in the average rural population. Nigeria, has a population of about 70 million of whom about 75% live in rural areas, so the reservoir of resistant strains may be one of considerable proportions, if the hypothesis is sustained.

In the current study, 53 strains (66.25%) had a reduced sensitivity to penicillin but 13 strains (16.25%) had high level resistance with MIC values over 0.6 µg penicillin per ml (Figure). This alarming finding supports the suggestion of Verhagen *et al.* (1971) that more high level relatively resistant strains occur in developing countries than elsewhere.

We did not test the sensitivity of the strains isolated in this study against the commonly misused antibiotics—such as, sulphonamides, streptomycin, tetracycline, ampicillin, and chloramphenicol—but judging by experiences elsewhere (Verhagen *et al.* 1971), it is probable that many of the strains isolated here would demonstrate multiresistance to antimicrobials readily available without prescription here, as in many developing countries. It is hoped that governments will be able to control the availability of these drugs to the public, if only to check the rapid sequential selection of resistant strains.

None of our strains produced β -lactamase. This is surprising as the β -lactamase gene is plasmid-borne. If it is assumed that this plasmid is transferred from other bacteria in an area of intensive penicillin and ampicillin use, one would expect that the indiscriminate use of antibiotics would give an environment favouring such genetic change. However, the number of our isolates from a single clinic may not be representative of the gonococcal population in

the whole country. The continuous surveillance of β -lactamase production by the gonococcus is obviously a priority in Nigeria as in many developing countries, since the cost and availability of other antibiotics may create considerable problems in treating cases, with resultant failure of control measures; these at present, are inadequate in many developing countries.

There is therefore a need to screen rural communities in tropical Africa to determine the extent of the reservoir of gonococcal infection and to ascertain the sensitivity pattern of the local strains to the commonly available antimicrobial agents. Financial constraints and inadequate facilities are common in these areas, and these together with promiscuity, polygamy, and ignorance constitute the major reasons for failure to control the rising incidence of gonococcal infection in many developing countries.

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